Supplemental Material: Section A.

Massachusetts Department of Environmental Protection (MA DEP) Scientific Advisory Committee on Health Effects of Perchlorate (SAC)

The MA DEP Scientific Advisory Committee on Health Effects of Perchlorate (SAC) was comprised of an independent advisory committee, augmented with a number of specialists with expertise in subject areas covered in the Department's assessment of the health effects of perchlorate. The SAC provided input and peer review of the MA DEP perchlorate toxicology assessment and RfD development.

The generous commitment of time and tremendous expertise of the SAC members was extremely helpful to MA DEP's efforts on perchlorate. MA DEP also wishes to acknowledge two members of the National Research Council Committee to Assess the Health Implications of Perchlorate Ingestion - Dr. Robert Utiger (Harvard University School of Medicine, Boston, MA) and Dr. Rosalind Brown (Children's Hospital, Boston, MA) - for sharing information and perspectives on the toxicity of perchlorate with MA DEP and the SAC. MA DEP is also grateful for the valuable input and information provided by Charles Emerson, MD, endocrinologist, University of Massachusetts Medical Center, Worcerster, MA.

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Supplemental Material: Section B.

Summary of Clinical Studies on Perchlorate.

Six controlled clinical studies (Brabant et al. 1992; Brabant 1994; Braverman et al. 2006; Greer et al. 2002; Lawrence et al. 2000 and 2001) have been conducted in which iodine sufficient adults were given perchlorate orally. These are summarized in Table S1.

Brabant et al. (1992) administered 13 mg/kg/day of potassium perchlorate orally for four weeks to five healthy male volunteers pretreated with 200 µg/day iodine for four weeks. No effects on thyroid volume or hormone levels were observed. However, intrathyroidal iodide concentration and serum levels of TSH decreased significantly, and serum levels of thyroglobulin nearly doubled. The authors speculated that the TSH decrease, which is the opposite of the expected response, might be an early adaptive mechanism to the iodine deficiency induced by perchlorate. This study identified a LOAEL of 13 mg/kg/day for thyroid-related effects. Brabant (1994) (unpublished data, communicated to U.S. EPA 2002a) repeated this study using longer perchlorate treatment and reported that thyroid volumes increased in all subjects, suggesting that treatment duration may increase perchlorate's effects.

Lawrence et al. (2000) treated nine healthy, iodide sufficient, adult male subjects with 0.14 mg/kg/day perchlorate orally for 14 days. Highly significant decreases in thyroid radioactive iodide (^{123}I) uptake (RAIU) were measured. The LOAEL for radioactive

iodide uptake inhibition (IUI) was 0.14 mg/kg/day. In a subsequent study, Lawrence et al. (2001) treated 8 healthy subjects with 0.04 mg/kg/day perchlorate and observed a non-statistically significant decrease in RAIU.

In another study, thirteen iodine sufficient volunteers (8 women and 5 men, 4-5 people per dose group) were given placebo, 0.007 or 0.04 mg/kg/day perchlorate orally for six months (Braverman et al. 2006). No perchlorate dose-related effects in TSH levels, averaged across the whole study group (male and females), were reported. Potential differences in responses by gender were not addressed.

Greer et al. (2002) administered perchlorate in drinking water at 0.007, 0.02, 0.1, or 0.5 mg/kg/day to 37 iodine-sufficient healthy male and female volunteers for 14 days, and measured IUI at different time points. Statistically significant IUI was observed in the three highest dose groups. IUI was also observed in the lowest group (0.007 mg/kg/day) but was not statistically significant. The low dose group was comprised of only 7 subjects and the individual relative uptake values exhibited considerable variability. The authors reported a study No Observed Effect Level of 0.007 mg/kg/day and estimated no iodide uptake inhibition levels based on a regression analysis, of 0.0052 and 0.0064 mg/kg/day for 8 and 24 hr RAIU, respectively. The 95% upper confidence limits on IUI at the true no effect levels ranged from 8.3 - 9.5%.

In summary, all of these clinical studies included small study populations, limiting their overall statistical power to detect effects. None of the studies addressed susceptible

populations including iodine insufficient women and children. The Greer et al. (2002) study was deemed to provide the best available dose-response data and was selected by MA DEP, as well as the NRC and CA EPA, as the key study in the derivation of an RfD for perchlorate (MA DEP 2006; NRC 2005; Ting et al. 2006).

Table S1. Comparative Summary of Clinical Perchlorate Exposure Studies

Study	N	Daily dose mg/kg/day	Exposure duration (weeks)	Effects RAIU	Total Serum T4	Free Serum T4	Total Serum T3	Free Serum T3	TSH
Brabant et al.1992 [b]	5♂	13 [a]	4	-	O	-	-	O	↓
Brabant pers. comm. 1994 [c]	NR	12	>4	-	-	-	-	-	O
Lawrence et al. 2000	9♂	0.14 [a]	2 + 2 follow-up	↓	-	O	O	-	O
Lawrence et al. 2001	8♂	0.04 [a]	2 + 2 follow-up	↓ [d]	O	-	O	-	O
Greer et al. 2002	7	0.007	2 + 15 day follow-up	↓ [d]	O	O	O	-	O
	10	0.02	As above	↓	O	O	O	-	O
	10	0.1	As above	↓	O	O	O	-	O
	10	0.5	As above	↓	O	O	O	-	↓
Braverman et al. 2006	4	0	24 + 4 follow-up	O	O	-	O	-	O
	5	0.007 [a]	As above	O	O	-	O	-	O
	4	0.04 [a]	As above	O	O	-	O	-	O

Key: O = no effect observed; ↓ = decrease in value of variable; NR = not reported; - = no observations reported; RAIU = radioactive iodine uptake; T3 = triiodothyronine; T4 = thyroxin; TSH = thyroid stimulating hormone

[a] Calculated by MA DEP assuming 70 kg body weight.

[b] Subjects pretreated for 4 weeks with 0.2 mg/day of iodide.

[c] Cited in U.S. EPA, 2002. Not peer reviewed. Thyroid volumes increased following perchlorate exposure.

[d] Non-statistically significant decline.

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Supplemental Material: Section C.

Summary of Key Animal Studies on Perchlorate Toxicity

Background

Due to a lack of adequate human data on the toxicity of perchlorate in sensitive subgroups, various rodent studies have been conducted to fill data gaps (U.S. EPA 1998; 2001). MA DEP's review of key animal studies, completed as part of its' weight-of-the-evidence assessment of perchlorate risks, is briefly summarized below.

Justification for Use of Animal Data in the Weight-of-the-Evidence Toxicity Assessment and RfD Derivation

MA DEP considered the rodent studies as useful supporting evidence in the perchlorate risk assessment process because: (1) the rat may provide a good model to represent sensitive subgroups, especially the iodine deficient pregnant woman and the congenitally hypothyroid fetus, which is largely dependent on maternal thyroid hormones during *in utero* development; (2) animal bioassays allow sensitive subgroups (the pregnant rat, fetus and neonate) to be directly tested; and, (3) the observed results in the animals are consistent with the proposed mode-of-action of perchlorate, including iodide uptake inhibition in the thyroid, thyroid and pituitary hormone perturbations and thyroid and brain structural alterations.

Studies Conducted in Animals at Different Life Stages

In summary the studies of perchlorate toxicity in animals (rats) have demonstrated the following effects:

- iodide uptake inhibition in the thyroid (Yu et al. 2000);
- decreased serum T4 and T3 levels and increased TSH levels (Caldwell et al. 1995; Argus Research Laboratories 1998a; 1998b, 2001);
- thyroid hypertrophy and hyperplasia across life stages, and tumors in F1 generation rats (Caldwell et al. 1995; Argus Research Laboratories 1998a, b)
- alterated brain morphometry and behavior in rat pups exposed *in utero* and after birth (Argus Research Laboratories 1998b; Bekkedal et al. 2000). Note: The NRC concluded that rats are more sensitive than humans to thyroid function disruption, which could account for the observed tumors. Perchlorate was found to be a potent promoter of thyroid tumors in animals (Hiasa et al. 1987) and to be nongenotoxic in various *in vitro* and *in vivo* studies (ManTech Environmental Technology 1998; Zeiger 1999), suggesting that the mechanism of tumor formation may be perturbation of thyroid and pituitary hormone homeostasis. A number of epidemiology studies (Pendergast et al. 1960; Pettersson et al. 1996; Wahner et al. 1966; Williams et al. 1977; Williams 1985; Vigneri et al. 1998) conducted in various parts of the world suggest that

iodine deficiency may increase the incidence of thyroid malignancy and alter the type of cancer produced. Since perchlorate can contribute to functional iodine deficiency, it may contribute to thyroid tumor risk if doses are sufficiently high, alone or in aggregate with other thyroid toxicants, to disrupt thyroid homeostasis and structure.

Key Study and Effect

MA DEP selected the Argus Research Laboratories (2001) study, which investigated sensitive endpoints at different life stages, as the key study. In this study, perchlorate exposures were associated with changes in pituitary and thyroid hormone levels and altered thyroid morphometry at different life stages, which were selected as the critical effect in the derivation of an animal data based RfD. Brain mophometry changes were also reported, but these have been questioned due to experimental design and execution issues. The study results are summarized below.

Thyroid and Pituitary Hormone Alterations and Changes in Thyroid Structure

In the key study, female Sprague-Dawley rats were treated with ammonium perchlorate at 0, 0.01, 0.1, 1.0, or 30 mg/kg/day in drinking water two weeks prior to cohabitation and continuing through the day of sacrifice. F1-generation pups were not directly dosed but might have been exposed *in utero* during gestation and via maternal milk and water during the postpartum period. Significant changes were observed in thyroid and pituitary

hormone levels at the lowest dose tested in different life stages. Thyroid histopathology was also altered in both dams and pups, although at higher doses. Although there is uncertainty as to the significance of the observed hormone changes, small and transient changes in thyroid hormone levels in pregnant women have been associated with neuropsychological impairments (Haddow et al. 1999; Klein et al. 2001) and, in rats, with structural changes in the brain (Argus Research Laboratories 2001; Auso et al. 2004). MA DEP elected to consider these changes in thyroid hormone status as an adverse effect.

Brain Morphometry Changes

Because thyroid hormones are important for normal neurodevelopment, the Argus Research Laboratories, 2001 study also investigated treatment-related changes in brain morphometry. Significant changes, especially in the corpus callosum and striatum, were observed at the lowest dose. Similar changes in various brain regions, especially in the corpus callosum and striatum, were also observed in the Argus Research Laboratories, (1998 a) study. However, various reviewers of the 2002 U.S.EPA perchlorate toxicity document have challenged the brain morphometry data, especially the plane of sectioning of the corpus callosum, leading to considerable uncertainty in the quantitative interpretation of this data. The NRC also reviewed several studies to establish biological plausibility of possible effects of perchlorate on the corpus callosum and concluded that, although not widely recognized as a classic marker of neonatal hypothyroidism, increased thickness of the corpus callosum appears to be a biologically plausible effect (NRC

2005). Based on the NRC perchlorate report (NRC 2005) and input from the MA DEP SAC, MA DEP concluded that the brain morphometry data should not be quantitatively used in the derivation of an RfD. That data does, however, qualitatively support concern over perchlorate neurodevelopmental toxicity.

Summary

Perchlorate treatment in rats produces changes in thyroid hormone levels, thyroid morphometry and thyroid tumors consistent with its proposed mode-of-action. The Argus (2001) study was used as the primary basis for deriving RfD values based on animal study data. MA DEP used information from this study as supporting evidence in its weight-of-the-evidence approach, rather than the primary point of departure for a final RfD. Due to the controversy surrounding the brain morphometry data, MA DEP concluded that the significant changes in thyroid and pituitary hormone levels observed in response to perchlorate were preferable endpoints and treated these effects as adverse. The lowest observed adverse effect level (LOAEL) for this endpoint was 0.0085 mg/kg/day (ClO_4^-). This value was used as a POD to derive animal-based perchlorate RfDs, as discussed below, for comparison to the values derived using human data from clinical studies.

Derivation of RfD Values Based on the Animal Data

MA DEP derived a range of animal-based RfD values using total uncertainty factors of 100 and 300, applied to the LOAEL dose in the Argus Laboratory study (2001). MA DEP and the MA DEP SAC concluded that UFs in this range were appropriate to account for LOAEL to NOAEL adjustment (UF = 10) and variability in human sensitivity and interspecies extrapolation (UF = 10 or 30). An UF of 1000, which is sometimes used to account for dose extrapolation (UF = 10), cross-species extrapolation (UF = 10) and human variability in sensitivity (UF = 10), was not used in this case, as sensitive life-stages were included in the animal studies and the animal model used may be particularly sensitive to perchlorate (NRC 2005). Applying a total UF of either 300 or 100 to the study LOAEL of 0.0085 mg/kg/day yields RfD values of 2.8×10^{-5} mg/kg/day and 8.5×10^{-5} mg/kg/day, respectively.

Conclusion

The range of RfDs derived based on the animal data span the final MA DEP RfD of 7×10^{-5} mg/kg/day based on human clinical data.

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